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THIN-LAYER CHROMATOGRAPHIC COMPARISON OF COMMERCIALLY AVAILABLE SILICA GEL COATED FILMS AND SHEETS WITH SILICA GEL COATED GLASS PLATES, DEMONSTRATED ON TOXICOLOGICALLY INTERESTING SUBSTANCES

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SUMMARY

Chromatographic data derived from silica gel layers on flexible films and glass fiber sheets are not always comparable with such derived from silica gel coated glass plates in given solvent systems. The presently available films and sheets with silica gel layers are compared with handcoated silica gel plates on ten selected acidic, neutral and basic drugs of toxicological interest, in two representative solvent systems. Reported are running rates, relative running rates, solvent mobilities and general characteristics of the different media.

INTRODUCTION

With the rapidly spreading popularity of thin-layer chromatography (TLC), a variety of commercial products have appeared on the market, to relieve the analytical chemist of the necessity to prepare his own plates. These are precoated glass plates, flexible supports of either plastic or metal (hence referred to as films) and glass fiber sheets (ChromAR and ITLC) which have the silica gel incorporated into the support (in the following referred to as sheets).

The comparison of the chromatographic properties of the presently available flexible supports with established data derived from silica gel coated glass plates, is the aim of this study. In order to limit the scope of the testing program, only silica gel layers without fluorescent indicators were compared. One exception had to be made with ChromAR sheets which are supplied only with fluorescent indicators.

The assumption that the support used for the silica gel layers is of no interest to the partition and/or absorption effects is obvious. However, in order to make the coatings more durable, "hardeners" are usually incorporated into the layers, the nature of which is a trade secret. The layers in such coatings, however, are not silica gel containing 10–15% calcium sulfate, but matrices with compositions which may alter the characteristics of chromatograms in given solvent systems. The importance of differences in effects and performance and the validity of extensive data files de-

rived from glass plates coated according to STAHL[1] with silica gel had to be researched if the replacement with the often more convenient films or sheets is to be considered.

The first company to appear with such a film was, to my knowledge, Distillation Products Industries, Division of Eastman Kodak Co., Rochester, N.Y., which marketed Chromagram. I have tested and compared this film with handcoated glass plates in three different solvent systems on toxicologically significant substances in 1967[2]. The code number and apparently the composition of the layer have since been changed.

A comprehensive study of a comparison of TLC adsorbents, supports and developing units was recently published in this journal[3]. While it is primarily dealing with handcoated and commercially coated glass plates it does include two films (Brinkman Instruments Inc. and Eastman Kodak Co..) and the glass fiber sheet of Gelman Instruments Co. Although it is not clear which of the Eastman Kodak and Brinkman films are used and the developing systems employed for toxicologically significant substances are different from this report, valuable comparisons are possible. It is solely for this reason of comparison that the columns of "Range" (difference in R_F values between the lowest and highest spots) and "Median" (median R_F value) are also used in this publication. It must be emphasized, however, that the resolution of a system (Range) and the relative position of the band of substances in the chromatograms (Median) are dependent on the choice of test substances.

EXPERIMENTAL

Solvents and standards

All organic solvents were of reagent grade and redistilled. The drug standards, made from pharmaceutical grade chemicals, were 0.5% (w/v) solutions in ethanol. The chromatograms were spotted with five microliter (25 μg) of standard, using disposable micropipets (Drummond Scientific Co., Broomall, Pa.).

Adsorbents

Silica Gel G for TLC according to STAHL: E. Merck A.G., Darmstadt, G.F.R. Distributed by Brinkman Instruments Inc., Westbury, N.Y. 20 × 10 cm glass plates were coated with 250 μ layers of silica gel using the Unoplan applicator with pneumatic alignment base (Consolidated Laboratories Inc., Chicago, Ill.).

Polygram Sil S-HR: Macherey, Nagel & Co., Düren, G.F.R. Distributed by Brinkman Instruments Inc.

Chromagram 6061: Distillation Products Industries, Division of Eastman Kodak Co., Rochester, N.Y.

Bakerflex Silica Gel IB: J. T. Baker Co., Phillipsburg, N.Y.

Ready plastic sheets for TLC: Schleicher & Schüll, Keene, N.H. (subsequently abbreviated as S & S film).

ChromAR sheet 500: Mallinckrodt Chemical Works, Laboratory Products, New York, N.Y.

Precoated TLC sheets, silica gel without fluorescent indicator, on aluminum: E. Merck A.G., Darmstadt, G.F.R. Distributed by Brinkman Instruments Inc. (subsequently abbreviated as EM film).

ITLC type SG: Gelman Instrument Co., Ann Arbor, Mich.

Developing unit

Rectangular glass jars, 22 × 11 × 23 cm, with sealed glass lids, covered with iron weights. All sides of the tanks were lined to a height of 18 cm with filter paper. The chambers were solvent saturated for a minimum of 6 h before use ("saturated tanks").

Developing systems

System 1: Chloroform–*n*-butanol–ammonia (70:40:5). System 2: Benzene–dioxane–ethanol–ammonia (50:40:5:5).

Details are reported[2]. The selection of drugs, all of toxicologic significance, was designed to cover the entire running distance from start to front. Practical considerations were in this respect subordinated to graphic considerations. Solvent system 1, for instance, would not be recommendable for the identification of Doriden in biological specimen. Since Doriden is a front runner, pigments in the frontal area might obscure its presence. Solvent system 2 would be a better choice in such instances.

Chromatographic procedures

Handcoated glass plates and the respective films and sheets were activated for 30 min at 110° and desiccated until used. Samples were applied with microcaps 3 cm from the bottom edge, 1.5 cm apart. The running distance was 15 cm. Spray reagents and spraying techniques were as described[2]. Barbital and codeine served as reference standards in the respective systems.

Standard conditions

Temperature throughout, 21 ± 2°; relative humidity, 40 ± 5%; mobile phase, 300 ml in "saturated tanks". Within the ranges of temperature and relative humidity no measurable effects on the running rates were observed in both systems.

Five substances in each group were analyzed on the eight supports. Runs where the reference standard did not come within R_F ± 0.05 of the average, were rejected.

RESULTS AND DISCUSSION

The averages of ten duplicate and acceptable determinations of each substance on each material in the specific system are listed in Table I as $R_F \times 100$ and $(R_F)_r \times 100$. The latter are based on experimental figures.

The solvent mobilities on the different media in the two solvent systems over a running distance of 15 cm are listed in Table II in cm/min together with mobilities relative to the fastest running medium in each system.

System 1

With the exception of ITLC, all materials gave a similar resolution of the given drugs in this group, expressed in the Range and Median figures. However, significant differences in R_F's and relative R_F's do exist: Bakerflex IB alone does not deviate more than *ca.* 0.05 R_F from the silica gel plates, which is the acceptable difference in our routine work. The S & S film is a very close second which only in the front region

TABLE I

$R_F \times 100$ VALUES OF ACIDIC, NEUTRAL AND BASIC DRUGS, DEVELOPED IN SATURATED TANKS, IN TWO DIFFERENT SOLVENT SYSTEMS

System 1	*Salicylic acid*		*Phenobarbital*		*Barbital*		*Secobarbital*		*Glutethimide*		*Range*	*Median*
	R_F	$(R_F)_r$	R_F	(R_F)	R_{Fr}	$(R_F)_r$	R_F	$(R_F)_r$	R_F	$(R_F)_r$	R_F	R_F
Glass plate	4	12	18	61	30	100	55	181	98	326	94	41
Polygram	5	19	16	65	25	100	46	186	88	355	83	36
Chromagram	7	18	25	67	38	100	62	164	90	239	83	44
Bakerflex	5	15	22	65	33	100	57	171	93	280	88	42
EM film	3	15	13	60	21	100	40	190	87	412	84	33
S & S film	5	15	19	63	29	100	51	176	86	297	81	38
ChromAR sheet	8	16	37	73	50	100	73	146	97	192	89	53
ITLC-SG	59	68	81	94	87	100	93	108	96	111	37	83

System 2	*Morphine*		*Codeine*		*Scopolamine*		*Meperidine*		*Methadone*		*Range*	*Median*
	R_F	$(R_F)_r$	R_F	$(R_F)_r$	R_F	$(R_F)_r$	R_F	$(R_F)_r$	R_F	$(R_F)_r$	R_F	R_F
Glass plate	9	27	33	100	45	134	66	197	77	232	68	46
Polygram	12	32	38	100	46	121	65	172	74	195	62	47
Chromagram	19	36	52	100	59	113	74	142	79	152	60	57
Bakerflex	12	32	38	100	48	126	66	173	75	197	63	48
EM film	8	28	29	100	38	131	56	192	65	227	57	39
S & S film	10	30	30	100	40	135	54	178	62	208	52	39
ChromAR sheet	25	44	57	100	69	121	82	143	89	154	64	64
ITLC - SG	84	89	94	100	96	102	97	103	98	104	14	94

BLE II

VENT MOBILITIES ON GLASSPLATES, FILMS AND SHEETS IN TWO SOLVENT SYSTEMS

	Glass plate	*Polygram*	*Chromagram*	*Bakerflex*	*EM*	*S & S*	*ChromAR*	*ITLC*
stem 1								
vent mobility (cm/min)	0.25	0.25	0.19	0.15	0.13	0.15	0.48	0.52
lative mobility	48	48	36	29	25	29	92	100
stem 2								
vent mobility (cm/min)	0.36	0.39	0.29	0.24	0.20	0.23	0.58	0.48
lative mobility	62	67	50	41	34	40	100	83

deviates more than 0.05 R_F from the glass plates. Chromagram 6061 has generally higher running rates but this type compares much better with glass plates than the former Chromagram K 301 R2 (ref. 2). The ChromAR sheets run consistently higher than the glass plates, Polygram and EM films run lower. On ITLC-SG all five substances are crowded in the upper third of the sheet. A similar, although less pronounced, trend can be seen in the Median column (see ref. 3, p. 293), where the type ITLC-SA is used on acidic substances in the chloroform–acetone system which the Gelman Industries Co. recommends.

In regard to resolution, the following classification in descending order can be seen: Glass plates, ChromAR, Bakerflex, EM, Polygram and Chromagram, S & S, ITLC. The smallest spots were obtained on glass plates and S & S films, closely followed by Bakerflex and EM. Chromagram had the largest spots.

The background color, although heavily depending on the spraying technique, was under comparable application the lightest on glass plates, followed by S & S, ChromAR, Bakerflex, EM, Chromagram and Polygram. The fading of the background color in artificial light or UV radiation was quickest and most complete on glass plates, S & S films and ChromAR sheets. It was quite good on Polygram, EM and ITLC and took the longest time and faded only incomplete on Bakerflex IB.

The running time over a distance of 15 cm was the shortest for ITLC, followed closely by ChromAR. The rest of the matrices is much slower running in the following, decreasing order: glass plates and Polygram, Chromagram, Bakerflex and S & S, EM.

System 2

Again with the exception of ITLC all materials gave similar resolutions. In this system, Bakerflex IB and Polygram have identical R_F's for the given substances and do not deviate more than *ca.* 0.05 R_F from the silica gel coated glass plates. Chromagram 6061 and ChromAR run consistently higher than glass plates. Chromagram 6061 compares again better with glass plates than the old type K 301 R2 (ref. 2). The EM and S & S films run lower than glass plates. On ITLC sheets the crowding of the substances in the frontal region was even more pronounced than in system 1 and no real comparison is possible.

The resolution in approximately falling order is: glass plates, ChromAR, Bakerflex, Polygram, Chromagram, EM, S & S, ITLC. The smallest spots were ob-

tained on glass plates with increasingly larger spots on Bakerflex, S & S, ChromAR, EM, Polygram and Chromagram.

The background color of the K_2PtI_6 stain was very light on glass plates, ChromAR, Bakerflex, S & S and EM films, somewhat heavier on Chromagrams and quite dark on Polygrams. In the latter case only a very light spray application prevented the background from obliterating the spots. The Dragendorff spray gives a lighter background and, though less sensitive, is recommended with Polygram films.

The running time over a 15-cm distance was the shortest for ChromAR followed by ITLC. The slower running layers in falling order were: Polygram, glass plates, Chromagram, Bakerflex, S & S, EM.

The most vulnerable layer is the handcoated silica gel layer on glass plates. All the other films were vastly superior to it. Some care was required not to tear the feltlike material of the ChromAR sheets. The ITLC sheets were found to be very brittle, particularly so after activation. The absorptivity of the coating, important for the speed of application of biological extracts, was best on ChromAR and ITLC, excellent on glass plates, good on EM, Bakerflex and S & S and only fair on Chromagram and Polygram.

In regard to solvent mobility the sheets compare very favorably with the rest of the materials. They are about twice as fast running as films and plates. The EM film is the slowest running in both systems and therefore, despite the short developing time in TLC, quite handicapped.

All the plastic materials were stiff enough to be self-supporting in the tanks and could be leaned against the wall or supported by metal racks. ITLC sheets were bending too much when gradually wetted by the solvent. ChromAR sheets were not self-supporting.

Since the developing solvents flow through the entire sheet in contrast to the surface floor of the other materials, glass plates cannot be used to support the two sheets in tank type containers. A completely spurious movement of the substances will occur in such attempts. Free suspension of the sheets from racks with metal clamps, with the ends hanging freely into the solvent systems is recommended, or as in the case of ITLC, the company manufactured developing apparatus.

REFERENCES

1 E. Stahl (Editor), *Thin-Layer Chromatography*, Springer Verlag, New York, 1969.
2 P. Schweda, *Anal. Chem.*, 39 (1967) 1013.
3 G. Kananen, I. Sunshine and J. Monfoote, *J. Chromatogr.*, 52 (1970) 291.